**Cloning M&M should include:**

Do not include ‘practice cloning’ on red/green/purple plasmids

Provide more detail than what was in your proposed methods from the Mid-Semester Research Report.

**NOTE**: When you list concentrations of reagents, you will almost always use the Final Concentrations of that reagent in the reaction volume (usually the final volume in the tube)

NCBI Gene #

Original sequence + Codon Optimized Sequence

These are called CDS (Coding DNA Sequences) vs. ‘genes’

Which organism is your sequence codon optimized for

Why do we use codon optimization?

**Cloning**

What is the overall purpose of the cloning?

pNIC-Bsa4 vector contents – what makes it useful to use

his Tag (expression vector), T7 promoter (what does it do)

(Company, Location) = (Structural Genomics Consortium, - the one in England)

How will you clone the gene into your expression vector? Why is one approach better than another? Our cloning method we go directly to expression vector instead of a cloning vector first. Can you use a cDNA clone (a plasmid vector) or genomic DNA of the organism?

Will you use antibiotic resistance – why?

Overlap PCR,

Explain Primary, Secondary, and PCR2 steps in general

Why is Overlap PCR advantageous over PCR from Genomic DNA?

Primer Design using DNA Works – codon optimized sequence

Talk about the primers and the Oligo mix – what is special about the primers?

Primers

List the sequences of ALL of your Oligo Primers

– designed in DNA Works but ordered from (IDT, Coralville, IA)

List the sequence of the special tail primers - (IDT, Coralville, IA)

FOR PCR

Final concentration of primers

Final MgCl2 concentration that worked

Final concentration of dNTPs

ng amount of starting plasmid template DNA

which polymerase and why? (Company, Location)

PCR cycling program (how many seconds and at what temperatures)

checked on a 1% agarose gel with Ethidium Bromide

Ligation Independent Cloning (cohesive end generation, cutting the accepting vector, annealing and transformation)

Cohesive end generation with cutting with BsaI first, then T4 DNA Polymerase

(What does the T4 Poly do? Which nucleotide did you add?)

PCR cleanup kit (Company, Location)

Annealing, transformation - type of cells (Company, Location)

Kanamycin and Sucrose plates - why? (SacB)

Miniprep to obtain DNA from clones

How do you check for positive clones?

NanoDrop - wavelength used for DNA (Company, Location)

Submit to DNA sequencing using which primers (list the sequence of the primers)

Midi prep - what is purpose? Which kit did you use? (Company, Location)

List (company, location ) e.g. Mini-prep kit (Sigma Aldrich, St. Louis, MO)

For each ‘non-common’ items only.

e.g. Agarose is common and the company, location do not need to be cited.

NOTE: For Restriction Enzymes, antibiotic, MgCl2, and dNTPs you don't need company info – because they are too commonreagents

For Final Paper you will need to CITE:

DNA WORKS site

For these Database pages, they usually have a Reference listed on their webpage that you can copy

Put your name in the Header of EACH page

Use page Numbers: p. 1, p. 2, p. 3 etc.

Justify your margins to full width (i.e. block style paragraphs)

SUBMITTING

Title your filename appropriately – using the standard format that we have been using.

**UTEID\_Name\_Date\_AssignmentName.doc**

Convert your document to a PDF

Upload to the **AssignmentsVDS** directory on the Liberty box (Linux machine). This is a write-to **only** directory. So, you won’t be able to see anyone else’s files that have been dropped there.

Use Secure Shell or WinSCP to login (similar to logging into the DDFE)

Host Name: **vdsclass.dyndns.org**

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